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EFFECTS OF WOUND BACTERIA ON POSTBURN ENERGY METABOLISM

ANNUAL/FINAL REPORT

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SUMMARY

The hypothesis examined in this study was that a major portion of the increase in resting metabolic rate (RMR) following thermal injury is initiated by bacteria and/or their products in the burn wound. RMR and core temperature (Tc) of 400-600 g male Sprague-Dawley rats were monitored before and after a full-thickness, 30% total body surface burn. Burn wounds were seeded with 10^8 non-virulent P. aeruginosa at the time of injury or allowed to colonized spontaneously. Seeding the wound resulted in a reproducible localized, gram-negative wound infection (10^6 colony forming units/gram wound) for two weeks after injury. Seeded rats expressed a 20-40% increase in RMR on postburn days (PBDs) 3-4, 7-8, and 14-15, while unseeded animals experienced little or no change in RMR or Tc until the second postburn week. Endotoxin, released in the burn wound, was not responsible for the increase in RMR, because (a) rats seeded with gram-positive bacteria (10^8 S. epidermidis) were equally hypermetabolic, (b) continuous endotoxin infusion beneath the unseeded wound did not increase RMR, and (c) hypermetabolic rats were not endotoxemic. Burned rats were febrile on PBDs 1-2, but the hypermetabolism of seeded rats on PBDs 3-4 and 7-8 occurred at normal central body temperatures. Efforts to alter postburn hypermetabolism by improving humoral and cell-mediated immunity were unsuccessful. A reduction in wound inflammation through topical or systemic hydrocortisone treatment also had no measurable impact on RMR of the seeded, non-bacteremic rats. Interleukin-1 (IL-1) activity was uncovered in the serum of burned rats, but there was no relationship between serum IL-1 levels and the metabolic response to injury. A non-cytotoxic, low molecular weight (less than 10 kD), relatively heat stable (56 °C for 30 minutes), inhibitor of IL-1 (INH) was also identified in the serum of burned rats. The INH suppressed native rat and human monocyte derived IL-1 activities, but the inhibition was overcome by increasing IL-1 concentration. Plasma from burned rats contained less IL-1 activity and what appeared to be an IL-1 inhibitor of lymphocyte proliferation. Preliminary data suggest the plasma inhibitor was different from serum INH. Taken together, the data indicate that afferent mediators of postburn hypermetabolism originate in the wound in response to active bacterial growth. These mediators are non-thermoregulatory in nature and resistant to variations in immunocompetence. There does not appear to be enough "free" IL-1 in the circulation to act as a afferent mediator of postburn hypermetabolism. The biologic role of the INH remains undefined, but suggest that systemic IL-1 effects reflect the balance achieved between this cytokine and its circulating inhibitors.

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This study examined the hypothesis that postburn hypermetabolism is initiated by bacteria and/or their products in the burn wound. Resting metabolic rate (RMR) and core temperature (Tc) of rats were monitored before and after full-thickness, 30% total body surface burns. Wounds were seeded with non-virulent P. aeruginosa at the time of injury or allowed to colonize spontaneously. Seeding resulted in a reproducible, localized wound infection and a 20-40% increase in RMR for two weeks after injury. Unseeded rats experienced little or no change in RMR until the second postburn week. RMR and Tc were related, but burned nonbacteremic rats were frequently hypermetabolic without being febrile. Endotoxin released in the wound was not responsible for the increased RMR. Postburn hypermetabolism was unaffected by variations in wound inflammation, or changes in humoral or cell mediated immunity. Interleukin-1 (IL-1) activity was uncovered in the serum of burned rats, but there was no relationship between IL-1 level and RMR. A low molecular weight (<10 kD)

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inhibitor of rat and human IL-1 (INH) was identified in serum of burned rats. Plasma from burned animals contained a low molecular weight IL-1 inhibitor, but it appeared to be different from the serum INH. The data indicate that afferent mediators of postburn hypermetabolism originate in the wound associated with bacterial colonization. These signals are non-thermoregulatory in nature and resistant to changes in immunocompetence. IL-1 does not appear to be a circulating mediator of postburn hypermetabolism, but its biologic role is obscured by the presence of inhibitors.

FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or service of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).

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INTRODUCTION

The rise in energy expenditure following trauma varies with the extent of injury and reaches its greatest magnitude in the burn patient. A young, otherwise healthy patient with a 50% total body surface burn (TBSB) can have a resting metabolic rate twice normal with every major system in the body working at an accelerated rate.

Catecholamines are the principal efferent mediators of postburn hypermetabolism (1), but the afferent signals which initiate and sustain the increased sympathoadrenal activity are largely undefined. The basic relationship between wound size and the degree of hypermetabolism suggests that these signals originate in the wound, and there is good evidence that they travel via the circulation to affect central nervous system control of energy expenditure (2,3).

Historically, burn patients were thought to be hypermetabolic because they were cold (4-6). The increase in metabolic heat production was believed to compensate for the increased evaporative heat loss from the wound. Heat balance following thermal injury is further complicated by the fact that patients are febrile and have to produce more heat to maintain body temperature above normal. While the data collected on small burns supported this thesis, gradually more and more investigators began to question the significance of a thermoregulatory basis for postburn hypermetabolism. The problem with this concept developed when postburn hypermetabolism could not be eliminated by either blocking wound evaporative heat loss or raising ambient temperature into the patient's thermoneutral zone (7-10). Since the metabolic rate at thermoneutrality is minimal and independent of changes in ambient temperature (11), the extra metabolism at thermoneutrality must be considered the energy cost of injury.

In the past, investigators have separated the metabolic responses to burn injury into those occurring before and after bacteremia or sepsis. They do so in order to distinguish between the effects of injury and the superimposed infection. While this is an important clinical distinction, it leads to the concept that the non-bacteremic patient is "free of infection" (12) and fails to address any systemic metabolic effects of bacteria prior to their actual entrance into the blood stream. Since the wound is not sterile during the hypermetabolic phase of injury and infection alone produces metabolic and neuroendocrine adjustments similar to those in thermal injury (13), wound bacteria and/or their products may be important metabolic stimuli in the "uninfected" patient.

Bacterial contamination of the burn wound increases the metabolic rate of rats. This was first suspected when seeding 30% TBSB wounds with non-virulent bacteria raised the metabolic rate of non-bacteremic animals above that of unseeded, burned controls (14). The hypermetabolic effects of wound colonization appeared as a continuum, first evident as a 10-30% increase in resting oxygen consumption of non-bacteremic animals and progressing to 40-50% increases with wound invasion and systemic infection. Topical antimicrobial treatments of seeded wounds reduced the hypermetabolic response of non-bacteremic rats, but without the associated bacteriologic studies, it was impossible to know whether the consequences of topical wound therapy were the result of reduced wound colonization or some other systemic effect of the antimicrobial agent.

If bacteria initiate the metabolic response while confined to the burn wound, they must release, or cause the release, of humoral mediators. These afferent signals may be bacterial products (enzymes, toxins, etc.) or cytokines produced by host cells in response to bacteria or their products. Endotoxin is a prime candidate in either case, since it increases metabolic rate upon entering the circulation (15) and is also a potent inducer of cytokine production (16). Interleukin-1 (IL-1) and tumor necrosis factor (TNF) are two cytokines believed responsible for many systemic responses to injury and infection. The hypermetabolic effects of both of these cytokines remain uncertain, and to date no one has found measurable quantities of either IL-1 or TNF in the blood of burned animals or humans.

The hypothesis examined in this study was that a major portion of the increase in energy expenditure following thermal injury is initiated by bacteria and/or their products in the burn wound. This report will describe the conformation of this hypothesis and the efforts to uncover the afferent mediators of postburn hypermetabolism.

METHODS AND MATERIALS

General Study Design. The basic protocol involved measuring resting metabolic rate and colonic temperature of rats before and after full-thickness 30% total body surface burns. Variations in wound bacterial density were achieved by seeding some wounds with non-virulent microorganisms while allowing others to colonize spontaneously. Topical antimicrobial agents were not used in order to avoid the potential for systemic drugs effects. Quantitative wound cultures were performed to characterize the extent of colonization, and blood and spleen cultures were used to identify bacteremic animals. Since this study was designed to address the effects of localized wound infection, bacteremic or septic animals were not included in the data analysis.

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Animals. The animals selected for study were 4-7 month old, male Sprague Dawley rats (Hilltop, Scottdale, PA) weighing 400-600 grams. They were housed in individual cages and had access to food and water throughout the study. A 12-hour light/dark cycle was maintained, and ambient temperature ranged from 25-28 ^{o}C while the animals were not under study. Metabolic studies were performed at thermoneutrality - 30 ^{o}C before injury and 32 ^{o}C after injury.

Inventory of Specific Procedures. In an effort to minimize duplication of text, the specific procedural details were omitted from this manuscript. The following is an inventory of all procedures employed in this study and where a complete description of each can be located.

Procedure	Annual Report	Pages
1. Burn injury and wound seeding	I	7
2. Resting metabolic rate	I	7-8
3. Colonic temperature	I	9
4. Peritoneal temperature	II	8
5. Burn injury and wound seeding	I	7
6. Bacteriology	I	9
7. Endotoxin preparation, infusion, assay	I	9-10
8. Special diet	I	10
9. Data analysis	I	10
	II	11
10. Subcutaneous infusion of dead bacteria	II	8
11. Wound hydrocortisone treatments	II	9
12. Active immunization against NVP	II	9
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14. Cyclophosphamide treatments	II	10
15. Indomethacin treatments	II	10
16. Interleukin-1 assay	II	10-11
17. Interleukin-1 inhibitor assay	II	11

IL-1 and Inhibitor Activities in Serum and Plasma. The only procedure not described in previous annual reports is the comparison of the IL-1 and IL-1 inhibitor activities in serum and plasma. Aortic blood was drawn aseptically from unburned rats and burned, NVP-seeded animals on PBD 7 or 8. Quantitative bacteriologic study was performed on each specimen, and only non-bacteremic samples retained for analysis. Blood for serum samples was placed in glass tubes at room temperature for 30 minutes before centrifugation. Blood for plasma extraction was placed in glass tubes containing EDTA (1.5 mg/ml blood) and aprotinin (0.67 TIU/ml blood). This blood was centrifuged at 400g for 10 minutes. The plasma was removed without disturbing the buffy coat, aliquoted in 1.5 ml microfuge tubes and spun at 10,000g at 4 ^{o}C for one minute to pellet platelets. Serum and plasma samples were frozen at -20 ^{o}C until prepared for assay.

Pooled serum and plasma samples (2.5 ml) were fractionated on a sterile Sephacryl S-200 column (1.5 cm x 60 cm), and 40-50 individual fractions collected (molecular weight range from 4 to 30 kD). Samples were eluted with sterile PBS and the column calibrated with molecular weight standards. Human recombinant IL-1 beta (hrIL-1b, 0.6 u/ml) was added to 50 ul of each fraction and IL-1 activity in the fraction determined by the standard IL-1 assay (AR II, page 10,11). Fractions containing rat IL-1 increased the IL-1 activity of that fraction above the hrIL-1b baseline, while those containing an inhibitor to IL-1 suppressed hrIL-1b activity.

RESULTS*

Metabolic Effects of Wound Colonization. Seeding the burn wound with bacteria caused an abrupt increase in wound colony count (10^6 CFU/g by PBDs 3-4) which remained constant over the next two weeks (Figure 2, AR I, page 11). Associated with wound colonization was an increase in resting energy expenditure (Figure 3, AR I, page 12). In contrast, the unseeded wound colonized more slowly and reached a comparable level of colonization sometime during the second postburn week. During the first week, when the unseeded wound contained less bacteria than the seeded wound, unseeded animals maintained lower resting metabolic rates.

When burn wounds colonized in a natural, spontaneous manner, there was a significant correlation between wound bacterial count and the increase in resting oxygen consumption of burned, non-bacteremic rats (Figure 6, AR I, page 15). There was little change in metabolic rate of the burned rat until wound bacterial concentration reached 10^4/gram, suggesting that animals with sterile wounds would not be hypermetabolic. Variations in the data, however, revealed that factors other than the number of _viable_ bacteria affect the relationship between wound colonization and postburn hypermetabolism.

Wound Endotoxin as a Afferent Mediator. The increase in metabolic rate following NVP seeding and gram-negative wound infection was presumed to be the result of endotoxin released in the wound. This proved not to be the case, however. Rats with localized, gram-positive (_S. epidermidis_, SE) wound infections were just as hypermetabolic as those with gram-negative infections (Table 2, AR I, page 16). In addition, continuous subcutaneous infusion of NVP endotoxin beneath the unseeded wound (at 10 times the estimated maximum rate of endotoxin release in

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* In an effort to avoid unnecessary duplication of details, only a brief, general review of the data is provided. For a more detailed description of specific findings, the reader is referred to the annual report (AR I or II) where the data were originally presented.

the infected burn wound) failed to raise resting metabolic rate above that of the burned controls (Table 3, Figure 8, AR I, pages 17, 18). Finally, the hypermetabolic, NVP-seeded rats were not endotoxemic.

Effects of Dead Bacteria. Heat-killed NVP or SE bacteria were continuously infused $(10^7-10^8/hr)$ into the subcutaneous tissue beneath the unseeded burn wound for two weeks. This increased the metabolic rate above unburned controls, but not substantially above that generated by endotoxin infusion alone (Table I, AR II, page 13; Table 3, AR I, page 17).

Effects of Hydrocortisone Treatments. The goal of this experiment was to determine whether a reduction in wound inflammation and IL-1 release affected the metabolic response to localized infection. Systemic glucocorticoid administration has been reported to reduce the accumulation of inflammatory cells in the wound and the rate of IL-1 production by these cells (17-19). Since glucocorticoids are calorigenic, the dose administered must be large enough to achieve the desired wound effects without raising metabolic rate. NVP-seeded rats were treated with topical or systemic hydrocortisone. Wound histology revealed a reduction in inflammatory cell density, but many treated animals were bacteremic on PBD 8. Glucocorticoid administration had no effect on the metabolic response of the non-bacteremic, NVP-seeded rat, but treated animals (topical and systemic) lost more weight over the period of observation (15 g/day) than the untreated animals (8 g/day).

Effects of Immunization. If postburn hypermetabolism is a response to NVP bacteria in the wound, the next question was whether immunization against these microorganisms would alter the metabolic response to NVP-seeding of the burn wound. Three different immunization protocols were used before we were satisfied with the level of antibody production. Despite a substantial increase in antibody titer of the immunized rats, there was no significant difference in resting metabolic rate between immunized and unimmunized rats following injury (Table III, AR II, page 15). Likewise, there was no correlation between postburn hypermetabolism and antibody titer on PBD 8.

Effects of Cyclophosphamide and Indomethacin. Low dose cyclophosphamide and indomethacin have been reported to restore cell mediated immunity (CMI) in burned mice (20). To determine whether the immunosuppression accompanying burn injury affects resting metabolic rate, burned, NVP-seeded rats received continuous, subcutaneous infusions of either cyclophosphamide or indomethacin. The metabolic response to burn wound colonization was not altered by either drug over the two week period of observation (Table IV, AR II, page 15), but there was no evidence that either cyclophosphamide or indomethacin improved CMI of the burned NVP-seeded rat.

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Serum Interleukin-1. When serum from burned rats was fractionated, pooled, concentrated and dialyzed, IL-1 activity appeared in two pools (one containing molecules with molecular weights between 15 and 30 kD and the other containing molecules less than 15 kD). The 15-30 kD pool displayed a significant increase in serum IL-1 activity following burn injury (Figure 2, AR II, page 16). There was no difference in the IL-1 activity between seeded and unseeded rats, and, therefore, no relationship between serum IL-1 activity and energy turnover.

Serum Interleukin-1 Inhibitor. When the dialysis material from burned rat serum was concentrated and desalted, it depressed rat serum IL-1 activity in a dose dependent manner (Figure 3, AR II, page 17). The IL-1 inhibitor (INH) had no effect on IL-2 stimulation of IL-2 dependent CTLL-2 cells (Figure 4, AR II, page 17). It was not cytotoxic for the two lympnocytes cell lines used in the IL-1 assay. Finally, the suppressive effects of the INH were inversely related to the quantity of IL-1 present in the sample (Figure 5, AR II, page 18). In this case, the inhibition was overcome by adding increasing amounts of human, monocyte derived IL-1 beta.

Plasma IL-1 and IL-1 Inhibitor Activity. Figure 1 illustrates differences in IL-1 activity in pooled plasma and serum samples from three unburned controls (A) and three burned, NVP-seeded rats (B).
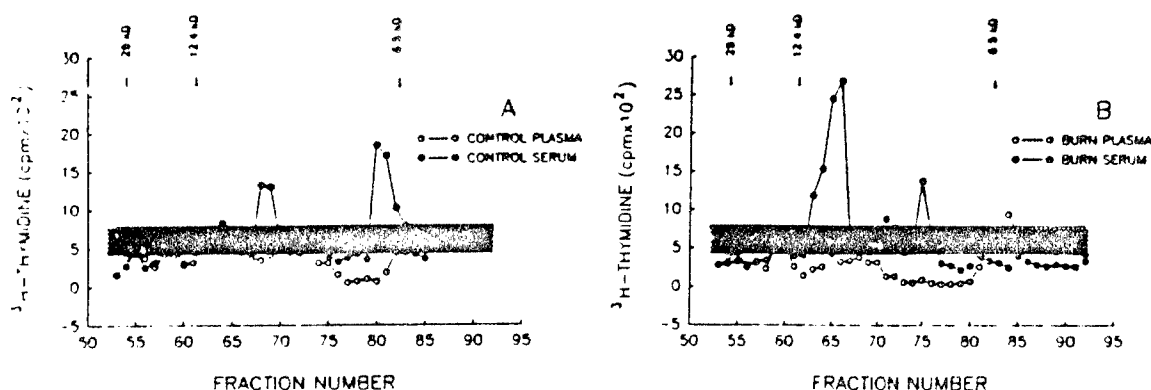
Figure 1. Fifty microliters of human recombinant IL-1 beta (hr IL-1b, 0.6 U/ml) was added to 150 ul serum and plasma fractions from unburned controls (A) and burned, NVP-seeded rats (B). IL-1 activity was assessed by lymphocyte proliferation (^{3}H-thymidine uptake in cpm). Shaded areas identify hrIL-1b activity (mean±SD). Fractions containing rat IL-1 added to baseline hrIL-1b activity while those with an inhibitor had a suppressive effect. Serum from control and burned rats contained IL-1 activity, while plasma from both groups had a low molecular weight inhibitor.

Serum from control and burned rats contained fractions with IL-1 activity, but there was no detectable IL-1 activity in either control or burn plasma. Conversely, there was little evidence of an IL-1 inhibitor in the serum of control or burned rats, but hrIL-1b activity was suppressed in numerous plasma fractions collected from both control and burned rats. The plasma inhibitor was the same relative size as the previously reported serum INH, but appeared to act differently. Unlike serum INH, the plasma inhibitor was (a) cytotoxic to LBRM cells, (b) heat stable at 100 ^{o}C, and (c) not specific to IL-1 since it blocked IL-2 stimulation of IL-2 dependent CTLL cells.

Changes in Core Temperature. Core temperature was monitored in two ways - (a) a single measurement from the colon one hour after each metabolic study and (b) continuous measurement from the peritoneum while the rat rested quietly in the afternoon (from 1300 to 1615 hours) and was active overnight (from 1800 to 0600 hours).

Colonic temperature (Tc) of the burned rat was normal or slightly elevated on PBDs 3-4 or 7-8. There was a tendency for the NVP-seeded rats to have higher Tc than unseeded rats (Figure 5, AR I, page 14), but frequently neither group was febrile on PBDs 3-4 or 7-8 (Table 2, AR I, page 16; Tables II and III, AR II, pages 14,15). In general, Tc of the non-bacteremic, NVP-seeded rat hovered in the high normal range from PBD 3 to PBD 8. It was not uncommon to find seeded and unseeded rats febrile during the second week after injury.

Peritoneal temperature of the burned NVP-seeded rat was frequently elevated at the beginning of the afternoon metabolic study (PBDs 3-4 and 7-8), but fell into the preburn range over the three-hour period of observation (Figure 1, AR II, page 12). Overnight temperatures exceeded afternoon values by roughly 1 ^{o}C before and after injury. This was associated with higher levels activity during the overnight study. Burned, NVP-seeded rats were consistently febrile over the first one or two days after injury. This increase in core temperature was evident in the afternoon and overnight and generally associated with a decrease in activity. This fever rapidly abated in most rats but reappeared in some animals by PBD 8.

DISCUSSION

Metabolic Effects of Localized Burn Wound Infection. This work supports the hypothesis that a major portion of the increase in energy expenditure following thermal injury is initiated by bacteria and/or their products in the burn wound. The correlation between wound colony count and resting oxygen consumption of burned, non-bacteremic rats demonstrates that bacteria exert systemic metabolic effects while still confined to the wound. Under normal conditions, bacterial contamination of

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the burn wound begins very soon after injury. This prevents an assessment of the hypermetabolic response to the injury alone, but all the data suggest that animals with sterile burn wounds would not be hypermetabolic. At what point wound bacteria begin to exert systemic metabolic effects is difficult to establish. In one study, unseeded animals were hypermetabolic on PBDs 3-4 when the average wound bacterial count was 10^4 CFU/gm (21), but there were many other unseeded burned groups which expressed no hypermetabolism on PBDs 3-4 or 7-8. The major increase in metabolic rate came in the second week when burn wounds contained 10^5-10^6 CFU/gm. Since the delay between injury and metabolic response could be reduced by seeding the wound, it appears that bacteria are essential components of the hypermetabolic stimulus. The important thing is not so much when the metabolic effects become apparent, but that they appear in a dose-response manner before microorganisms are discovered in the blood and the animal (or patient) is considered "infected".

Potential Afferent Signal(s) From the Colonized Burn Wound. I. Endotoxin. The correlation between wound colony count and the increase in resting oxygen consumption of non-bacteremic rats was significant, but variations in this relationship indicated that factors other than the number of viable bacteria in the wound also affected the metabolic response to thermal injury. Prior to this study, most would have speculated that endotoxin was the afferent mediator or stimulated the production of endogenous mediators of postburn hypermetabolism. Surprisingly, the data showed that endotoxin released in the colonized wound was neither an obligatory afferent mediator nor the major inducer of other mediators of postburn hypermetabolism in the non-bacteremic rat (22).

Humans are exquisitely more sensitive to endotoxin than rats, so the clinical significance of wound endotoxin cannot be derived from rat data alone. It is possible that species differences in endotoxin sensitivity explain the greater hypermetabolic response of humans with the same size burn (1,8). Even if this is the case, the rat data suggest that a sizable portion of the hypermetabolic response to wound colonization is not related to local release of endotoxin.

One explanation for the failure of endotoxin to reproduce the hypermetabolic response to gram-negative wound infection is that it is only one of many bacterial stimuli active in the burn wound. To administer only this one component of the cell wall may reduce the strength of the overall stimulus to a point that it is no longer effective. Chronic subcutaneous infusion of heat-killed bacteria proved equally ineffective suggesting that living bacteria and/or their products are more important hypermetabolic stimuli in the wound. Liu (23) has shown that P. aeruginosa produce several extracellular toxins significantly more potent than endotoxin. Since gram-positive seeded wounds caused rats to become as hypermetabolic as animals with gram-negative wounds, the proposed "active" components of the hypermetabolic stimulus do not appear to be specific to a particular bacterial strain.

II.   **Interleukin-1.**   Interleukin-1 (IL-1), a polypeptide produced by activated phagocytic cells in response to injury, infection, and antigenic challenge, is one of a host of chemical mediators released in the burn wound (24).   Upon entering the circulation, IL-1 acts like a "wound" hormone to initiate a wide variety of physiologic and immunologic responses.   C.A. Dinarello, a recognized leader in IL-1 research, states that "The best clinical example to illustrate the effects of IL-1 on the host is in patients with ongoing, localized bacterial infection" (25).   The stable, non-bacteremic burn patient is in this category and manifest most, if not all, of the systemic responses to IL-1.

The problem is that, while there is plenty of indirect evidence of systemic IL-1 activity following thermal injury, there is very little evidence that this cytokine is in the blood of burn patients. An endogenous pyrogen (original name for IL-1) was discovered in serum from burn patients (2), but it was never established as IL-1.   Clowes et al. (26) found a "proteolysis-inducing factor" in the plasma of three burn patients, which subsequently proved to be an active fragment of IL-1 (27). Initially, we found increased IL-1 in the serum of burned rats (28), but subsequently have discovered increasing amounts of IL-1 activity in the serum from unburned rats (unpublished data). Consequently, original differences between burned and unburned serum IL-1 levels have largely disappeared.   Cannon et al. (29) found IL-1 production was promoted by the in vitro clotting process and concluded that plasma IL-1 activity was a better index of circulating levels. We confirmed these findings in our burned and unburned rats when we uncovered IL-1 activity in the serum but not in plasma of the same animals.

It is reasonable to conclude that IL-1 is not an endogenous mediator of postburn hypermetabolism.   Drost, who found IL-1 activity in the serum of burned rats (23), was unable to do so in burn patients (personal communication).   Intravenous infusion of IL-1 has had mixed effects on metabolic rate of unburned rats (30,31).   Similarly, injections of etiocholanolone, an IL-1 inducer, in normal humans yielded indirect evidence for systemic IL-1 release without causing a change in energy expenditure (32). Finally, while postburn hypermetabolism is directly related to burn size, recent evidence suggests that there is an inverse relationship between burn size and plasma IL-1 levels (33).

The search for IL-1 lead to the discovery of a low molecular weight inhibitor of IL-1 in the serum of burned rats (34). This inhibitor (INH) reduced the capacity of rat and human IL-1 to induce IL-2 production by murine lymphoma cells.   While its mechanism of action is unknown, the capacity to overcome its suppressive effects by the addition of IL-1 suggests some form of competitive inhibition.   This may occur (a) by binding to IL-1 to block its active site, (b) by interfering with IL-1 receptor binding at the target cell or (c) by disrupting some post binding function of IL-1 in the target cell. All such mechanisms have been previously described for other IL-1 inhibitors (35-37).

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An IL-1 inhibitor of comparable size has been identified in the supernatant of cultured peripheral blood mononuclear cells (PMNCs) from healthy volunteers (38). The data suggest that the same cells produce both IL-1 and an IL-1 inhibitor. PMNCs collected from AIDS patients appear to make more of this IL-1 inhibitor than IL-1 (39). Due to the key role IL-1 plays in promoting both cell-mediated and humoral immunity, this imbalance between IL-1 and its inhibitor may contribute to the profound immunosuppression in the AIDS patient. Our data suggest that a similar inhibitor may be present in the blood of burn patients.

Serum from burn patients contains a small immunosuppressive factor (suppressive active peptide or SAP) (40), which is in the molecular weight range of the burn INH and the PMNC IL-1 inhibitor just described. While SAP has not been linked to IL-1, it acts to inhibit thymocyte proliferation by causing the production of PGE_2 (41). This is the same mechanism proposed for the PMNC IL-1 inhibitor.

We have uncovered a potent suppressive agent in plasma from burned and unburned rats (Figure 1). While this suppressor is the same relative size as serum INH, it did not appear to have the same biologic activity. The clinical implications of the INH and the plasma suppressor are unknown, but they could potentially include many immunologic and physiologic adjustments to injury. In some incidences they could be protective and in others quite destructive. Taken together, the IL-1 and inhibitor data indicate that the systemic effects of IL-1 are not determined by its rate of production or the amount which enters the circulation but rather by the balance achieved between IL-1 and its inhibitors.

Core Temperature and Energy Turnover. There is a general relationship between the increase in metabolic rate and the increase in core temperature of the non-bacteremic burned rat (21). For this reason, any search for afferent mediators of postburn hypermetabolism must include an evaluation of the thermoregulatory status of the model. If seeding the burn wound caused the rat to become febrile, then the increase in metabolic rate could be simply a thermoregulatory response to raise body temperature.

When large numbers of animals were studied, the average colonic temperature of seeded rats was statistically above those of unburned or burned unseeded animals (21). This difference is usually less than 0.5 °C and rarely evident when smaller groups are studied. Through the use of biotelemetry, we followed changes in peritoneal temperature of unrestrained rats over extended periods of time. There was a transient increase in peritoneal temperature of the non-bacteremic, NVP-seeded rat on PBDs 1 and 2, but it disappeared by PBD 3. On postburn days 3-4 and 7-8, peritoneal temperature was frequently above normal when the animals entered the metabolic chamber, but it usually fell into the normal range over the three-hour period of measurement. The elevated peritoneal temperature at the beginning of the

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metabolic study most likely reflected the response to handling superimposed on the basic hypermetabolic background.

Since, postburn hypermetabolism was commonly demonstrated at normal body temperature, the increase in metabolic heat production cannot be considered part of a fever response. Both thermal and non-thermal metabolic drives exist and contribute to the hypermetabolic state of the burned patient, but the non-thermal drive appears to be a more consistent response to localized burn wound infection. This separation and the relative significance of thermal and non-thermal metabolic drives have been described earlier in both patients and animals (8,10).

Resiliency of Postburn Hypermetabolism. We tried a number of different things in an effort to alter the metabolic response to NVP-seeding of the burn wound. These included (a) topical and systemic glucocorticoid treatments to reduce wound inflammation and IL-1 production; (b) low dose infusions of cyclophosphamide and indomethacin to improve cell-mediated immunity; and (c) active immunization against NVP. While there was no evidence of any improvement of CMI, we did achieve our other two goals.

Neither the reduction in wound inflammation nor active immunization against NVP had a measurable effect on postburn hypermetabolism. In the last annual report, I concluded that the inability to modify postburn hypermetabolism by these immunoregulatory techniques "demonstrated both the complexity of the afferent signal and the resiliency of the response." The complexity of the afferent signal becomes evident upon considering the host of interactive endogenous mediators released in the wound (IL-1 through IL-8, tumor necrosis factor, interferons, prostaglandins, etc.) and how each may be modified by various blood constituents.

The resilience of postburn hypermetabolism may have biologic implications or simply illustrate the "two-headed" nature of this experimental model. If, on the one hand, postburn hypermetabolism is a controlled response, then manipulations of one contributor (the immune system) may be compensated for by changes in other homeostatic mechanisms. Conversely, resiliency may be nothing more than a common response to different events. For example, the hypermetabolic response varies as a function of the degree of infection (21). If we reduce the inflammatory response of the wound and limit the production of inflammatory mediators, enhanced bacterial growth and wound invasion raise the metabolic rate. On the other hand, an improvement in humoral or cell-mediated immunity may accelerate and increase the release of endogenous mediators and promote postburn hypermetabolism. All we have done in this case is to replace exogenous mediators with endogenous mediators. If this is what is actually happening, the changing character of the afferent signal may be impossible to define with postburn hypermetabolism.

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CONCLUSIONS

The connection between wound colonization and postburn hypermetabolism of the rat is now firmly established, but the afferent mediators remain undefined. While the model is a localized, gram-negative burn wound infection, there is strong evidence that endotoxin or other components of the bacterial cell wall are not principal stimuli. Rather the data suggest that living microorganisms and their products are required for full development of the afferent signal. The increase in energy expenditure is not to maintain the febrile state, for it persist long after the acute febrile response has abated. The IL-1 present in the serum of burned rats appears to be a product of in vitro clot formation. The discovery of a low molecular weight IL-1 inhibitor offers yet another mechanism by which to control the systemic biologic and immunologic effects of this wound hormone.

RECOMMENDATIONS FOR FUTURE RESEARCH

There are two research goals established by the current project. They are:

I. Further characterization the chemical nature of the afferent signal(s) of postburn hypermetabolism. I would approach this problem in the following manner.

1. Collect subcutaneous fluid from beneath NVP-seeded and unseeded wounds and compare the hypermetabolic potential of these fluids with fluid from beneath normal skin.

2. Determine whether burned unseeded or unburned rats become hypermetabolic when infused subcutaneously with fluid collected from burned seeded rats. Burned unseeded and unburned rats receiving subcutaneous fluid from burned unseeded and unburned rats would serve as controls.

3. If so, separate water- and lipid-soluble components of the hypermetabolic fluid and repeat #2 with each component.

4. If not, concentrate the infusate and repeat #2.

5. If hypermetabolism cannot be transferred via subcutaneous infusion, determine the metabolic effects of intravenous infusion of the same material.

6. If IV infusion causes either the burned unseeded or the unburned rat to become hypermetabolic while the controls maintain normal metabolic rates, then repeat #3 and test water-soluble and lipid-soluble components in burned un-

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seeded and unburned animals.

7. If IV infusions from hypermetabolic rats do not raise the
    metabolic rate of burned unseeded or unburned rats,
    concentrate infusate and repeat.   If still unable to
    transfer a wound-derived hypermetabolic agent by IV
    infusion, one must conclude either that there is no such
    agent or the methodology altered its biologic activity.

8. If a hypermetabolic agent is located in either the
    water-soluble or lipid-soluble compartment of the
    subcutaneous fluid, begin processes of separation and
    identification.

II. Characterization of the low molecular weight inhibitor of
IL-1 in the serum and plasma of burned rats. I would approach
this project in the following manner.

1. Collect   aortic blood from   burned seeded,   burned
    unseeded and unburned rats.

2. Split blood samples into serum and plasma components.

3. Fractionate, concentrate, and dialyze serum and plasma
    samples and test for IL-1 activity in LBRM biologic
    assay.

4. Determine whether the low molecular weight inhibitor in
    plasma and serum are identical.

5. Determine the mechanism of IL-1 inhibition.   We know the
    serum inhibitor is specific for IL-1 and appears to act
    in a competitive manner. If the plasma inhibitor acts in
    a similar fashion, then the mechanism can be studied with
    either.   If the two inhibitors are different, we will
    have to approach each mechanism separately.   We will
    select the serum inhibitor, if the plasma inhibitor is
    not specific for IL-1.

The details of this protocol are provided in a proposal entitled
Characterization of a low molecular weight inhibitor of
interleukin-1 in the blood of thermally injured rats (USAMRDC Log
Number 89157002) currently under review.

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